Looping efficiency depends on the length of the intervening sequence with preference for a 15 nucleotides spacer or longer between the pyrimidine-tracts. RRMs 3 and 4 bind the 5′ and the 3′ pyrimidine-tracts, respectively, in a specific directionality, and work synergistically for efficient RNA looping in vivo.

386-Pos

Single-Molecule Imaging of DNA Curtains Reveals Intrinsic Energy Landscapes For Nucleosome Deposition

Mari-Liis Visnapuu, Eric C. Greene.
Columbia University, New York, NY, USA.

We employ nanofabricated diffusion barriers to organize DNA into molecular curtains allowing us to directly image thousands of aligned molecules and determine coarse-grained intrinsic energy landscapes for nucleosome deposition on model DNA substrates. Our results reveal distributions that are correlated with recent in silico predictions, reinforcing the hypothesis that DNA contains some intrinsic positioning information. We show that cis-regulatory sequences in human DNA coincide with peaks in the intrinsic landscape, whereas valleys correspond to non-regulatory regions, and we present evidence arguing that nucleosome deposition in vertebrates is influenced by factors not accounted for by current theory. We also demonstrate that intrinsic landscapes of nucleosomes containing the centromere-specific variant CenH3 are correlated with patterns observed for canonical nucleosomes, arguing that CenH3 does not alter sequence preferences of centromeric nucleosomes. However, the non-histone protein Scm3 alters the intrinsic landscape of CenH3-containing nucleosomes, enabling them to overcome the otherwise exclusionary effects of poly(dA-dT) tracts, which are enriched in centromeric DNA. In addition, these methods establish a platform that allows direct visualization of DNA binding proteins, DNA translocases and chromatin remodelers as they interact with single fluorescent nucleosomes and denser chromatin arrays.

387-Pos

Fluctuating Forces Facilitate Protein-Mediated DNA Looping

Yih-Fan Chen, J.N. Milstein, Jens-Christian Meiners.
University of Michigan, Ann Arbor, MI, USA.

Lac repressor-mediated DNA looping has become a paradigm for long-range genetic regulation. Our earlier experimental results have shown that forces on the order of a hundred femtonewtons can drastically disrupt the formation of DNA loops. This exquisitely high sensitivity to applied force implies that tension in the DNA, two orders of magnitude smaller than typical piconewton intracellular forces, may provide a mechanical pathway for transcriptional control.

We investigate how such mechanical switching is affected by fluctuating forces instead of static forces inside a cell. Our results show that by slightly increasing the magnitude of the fluctuations, which are on the order of tens of femtonewtons, the DNA loop formation rate can be significantly increased while the magnitude of the average tension in the DNA remains the same. This result contributes to our understanding of how protein-mediated DNA looping processes, which are extremely sensitive to force, can function in a noisy *in vivo* environment.

To study the effects of force fluctuations on DNA looping, a random series of optical forces displaying the statistics of Gaussian white noise is applied to a surface-tethered DNA molecule by axial optical tweezers. The lifetimes of the looped and unlooped states are measured under fluctuating forces that have the same average magnitude but different fluctuation strengths. Our results show that, as compared to the noise free case, the lifetime of the unlooped state decreases by about a factor of two when fluctuations on the scale of tens of femtonewtons are applied while the lifetime of the looped state remains constant.

388-Pos

On the Structure, Function and Metalloregulatory Properties of the Zinc-Activated Repressor *Streptococcus Pneumoniae* AdcR

Alfredo J. Guerra[1], Hermes Reyes-Caballero[1,2], Faith E. Jacobsen[1], Uma Mahendra Kumar Koppolu[3], Robert A. Scott[3,4], Krystyna M. Kazmierczak[5], Malcolm E. Winkler[5], David P. Giedroc[1].
[1]Department of Chemistry, Indiana University, Bloomington, IN, USA, [2]Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX, USA, [3]Department of Chemistry, University of Georgia, Athens, GA, USA, [4]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA, [5]Department of Biology, Indiana University, Bloomington, IN, USA.

A response to a change in transition metal ion concentration is mediated by metal-sensing transcriptional regulators that harbor metal-specific coordination sites. Zinc homeostasis in the gram positive human pathogen *Streptococcus pneumoniae* (*Spn*) is maintained by two novel zinc-regulated repressors, SczA and AdcR. *Spn* AdcR is the first putative metal-dependent member of the MarR family of transcriptional repressors. Expression profiling on BHI media under microaerobic conditions with a wild-type and isogenic Δ*adcR* strain reveals that AdcR regulates the expression of genes encoding the high affinity zinc uptake system *adcRCBA*, a group of zinc-binding pneumococcal histidine triad proteins (PhtA, PhtB, PhtD and PhtE) and an orphan AdcA homologue (AdcAII). Much of the *adcR* regulon is necessary for the virulence of *Spn*. Analytical ultracentrifugation experiments reveal that AdcR is a 32 kDa homodimer. X-ray absorption spectroscopy is consistent with a primary five-coordinate N/O regulatory site, a finding unprecedented for a zinc-sensing metalloregulatory protein. As expected, Zn(II) binding strongly activates *adc* operator DNA binding on the basis of quantitative fluorescence anisotropy assays (pH 6.0, 0.2 M NaCl, 25°C). Nearly complete backbone (H_N, ^{15}N, $^{13}C\alpha$, $^{13}C\beta$) resonance assignments of apo-AdcR (pH 6.0, 0.05 M NaCl, 35°C) reveal a highly α-helical two-fold symmetric homodimer, and that zinc binding perturbs resonances in the C-terminal regulatory domain, as well as the N-terminal winged helical DNA binding domain. Mutagenesis of at least two His in a highly conserved histidine-rich sequence in the regulatory domain (His108, His112), significantly modulates zinc regulation in vitro and in vivo. Progress on the solution structure and residue-specific dynamics of AdcR in the apo- and zinc activated states will be reported. Supported by NIH grants GM042569 (to D.P.G.), F32 AI084445 (to F.E.J.), GM042025 (to R.A.S.) and AI060744 (to M.E.M).

389-Pos

DNA Structure Specificity of Bacillus Stearothermophilus PcrA

Karen R. Thickman, Sanford H. Leuba, Saleem A. Khan.
University of Pittsburgh, Pittsburgh, PA, USA.

Helicases are molecular motors that play critical roles in nucleic acid transactions, including replication, repair, recombination, and transcription. As observed in a number of diseases resulting from mutations in helicase genes, helicases are important for maintenance of cellular functions. A feature of these diseases is an increase in malignancies as a result of genome instability resulting from unregulated DNA recombination. Gram-positive bacteria harbor a conserved helicase, PcrA, which is involved in UV-damage DNA repair, plasmid rolling-circle replication, and regulation of DNA recombination. PcrA has been shown to inhibit RecA-mediated DNA strand exchange reaction and displace RecA from the DNA. Homologs of PcrA, including Rep and UvrD helicases, have been shown to be 3′ to 5′ helicases. However, PcrA homologs from *Staphylococcus aureus* (*S. au.*), *Bacillus anthracis*and *Streptococcus pneumoniae*also exhibit 5′ to 3′ helicase activity. In these studies, we have explored the directionality and DNA structure specificity of *Bacillus stearothermophilus* (*B. st.*) PcrA. We have demonstrated that *B. st.* PcrA does not have 5′ to 3′ directionality on standard partially duplex DNA substrates containing a 5′ oligo dT tail. However, similar to *S. au.*PcrA, *B. st.* PcrA unwound DNA substrates with a hairpin structure found at the dsDNA replication origin in the rolling-circle replication plasmid pT181 with high efficiency. These included substrates with only a 5′ single-stranded region. These results indicate that though the *S. au.* and *B. st.* PcrAs are 60% identical, they have different activities. Our future work will explore the amino acid sequence differences in these helicases that lead to their differential biochemical activities.

390-Pos

Single Molecule Studies on Hcv RNA Polymerase Activity

Pierre Karam, Colins Vasquez, Wayne Mah, Megan Powdrill, Matthias Götte, **Gonzalo Cosa**.
McGill University, Montreal, QC, Canada.

NS5B is an RNA-dependent RNA polymerase capable of initiating RNA synthesis de novo. However, the detailed underlying mechanism remains elusive. It is unclear how the enzyme locates the 3′-terminus of the RNA template. Previous studies suggested that the nucleic acid binding channel of NS5B accommodates approximately 10 residues of a single stranded RNA. Although the contacts between the polymerase and its nucleic acid substrate are maximized, the 3′-end of the primer is not properly positioned under these conditions and such complexes are therefore unproductive. Hence, it is conceivable that the NS5B-RNA interaction is highly dynamic. Of note, nonnucleoside inhibitors of NS5B were shown to inhibit formation of a competent complex. To address this problem, we have conducted single molecule FRET (SM-FRET) experiments. This approach allowed us to obtain a direct visualization of both the positioning and dynamics of NS5B in complex with its RNA template. We performed our experiments on single -donor (Cy3)/acceptor (Cy5) fluorophore labeled- RNA substrates, which were surface-immobilized to enable long observation times. Binding of NS5B caused a significant increase in FRET. SM-FRET studies on RNA-protein complexes revealed protein sliding dynamics occurring in the millisecond time scale. These dynamics change with the RNA template length, and with the presence of complementary DNA strands that restrict the motion of NS5B. A nonnucleoside inhibitor is observed to compromise binding of NS5B to the template. Taken together, our single molecule studies provide direct evidence for the ability of NS5B to slide along its RNA template. Sliding of NS5B provides a plausible mechanism that facilitates